Remarks

Prior to entry of this amendment, claims 22-28 and 32-44 are pending in the application. By this amendment, the Specification has been amended to correct and update the priority claim, and to comply with requirements regarding use of a trademark. In addition, claims 22, 25, 35, and 44 are amended, claims 27 and 39-43 are cancelled, and new claims 45 and 46 are added. Support for claim amendments or new claims, if necessary, is discussed below. No new matter has been added by this amendment.

After entry of this amendment, **claims 22-26, 28, 32-38, and 44-46 are pending** in the application.

Objections to the Specification

In accordance with the Examiner's suggestions, Applicants have amended the specification to correct the form of the trademark "GENECHIP®" at page 2, and to add the generic terminology. Applicants have also updated and corrected the priority claim, which was originally added by preliminary amendment on July 25, 2003. Applicants thank the Examiner for bringing these issues to their attention.

Claim Objections

Claims 39-43 have been objected to by the Examiner under 37 CFR 1.75(c), on the ground that they do not add further limitation to the claim from which they depend (claim 22). To expedite prosecution of the current application, Applicants have cancelled claims 39-43, thereby rendering this rejection moot. Applicants note, however, that in no way is cancellation of these claims intended to limit the types of samples that can be analyzed using the kit of claim 22. In particular, the kit can be used to analyze any biological samples containing biomolecules, wherein the biomolecules have a relative relationship to each other in at least two dimensions within the biological sample. Examples of such types of samples include tissue sections, microarrays (for instance, which contains a plurality of DNA probes, antibodies or a combination thereof), gels, and microtiter plates.

In light of cancellation of claims 39-43, Applicants request that the objection to these claims be withdrawn.

Claim Rejections – 35 USC §112 2nd Paragraph

Claim 27 is rejected as being allegedly indefinite for use of the term "low." More specifically, it is alleged that this term is not defined by the claim, and that the specification does not provide a standard for ascertaining the requisite degree. Applicants contest the allegation that the term "low" is not definite. However, in order to expedite prosecution of the current application, Applicants have cancelled claims 27, thereby rendering this rejection moot.

Claim 38 has been amended as suggested by the Examiner, to correct antecedent basis for the term "said porous substrate." Applicants request this rejection be withdrawn as it is now moot.

Claim Rejections – 35 USC §102

Claims 22-25, 27, 28, 33-36, and 39-44 are rejected as allegedly anticipated by each of Christian, Nieuwkerk *et al.*, and Imai *et al.*, separately. Applicants traverse each of these rejections; specific arguments are provided below.

As a preliminary matter, it is alleged in regard to all of the anticipation rejections that it is "**inherent** that the membranes of the invention are under 30 microns in thickness" (*see* current Office action, at page 4, lines 8-9; page 5, lines 1-2 and 11-12. It is not clear whether the Office intended the phrase "membranes of the invention" to refer to the cited reference invention, or Applicants' invention, but in either case Applicants strenuously traverse this rejection.

Membranes come in many different thicknesses, and there is nothing whatsoever that would **necessarily require** (*i.e.*, make it an inherent feature) that the membranes of the invention, or of any cited reference, would be under 30 microns in thickness. By way of example, Applicants attach herewith as Appendix A several public documents available on the internet, each of which describes a commercially available membrane used in methods of

transferring or capturing biomolecules. Each of these documents indicates a thickness of the described membranes. Thicknesses found in a very cursory search of public sources include: 6.0 mils (152.4 microns), 147 microns, 145 microns, 152 microns, 140-250 microns. (To convert mils to microns (micrometers, μM), multiply the number of mils by 25.4.)

In addition, there is no explicit or implicit teaching of a membrane of less than 30 microns thickness in any of the cited references. The Office action does not point to any such teaching, nor have Applicants been able to identify any text in the cited references regarding a membrane thickness of less than 30 microns. The Office has not met its burden to demonstrate that this characteristic is inherent in any membranes whatsoever.

Applicants submit that there is nothing whatsoever **inherent** in a membrane for use in the current invention, or in the cited reference, that would require that it be less than 30 microns. Applicants request that the next action include a withdrawal of this statement.

Rejection Based on Christian (EP 0 139 373 A1)

All of the pending claims have been rejected as allegedly anticipated by Christian. As stated in the current Office action (in the paragraph bridging page 3-4), Christian describes an immunoassay rod that is "based on a column of spaced detection layers with each detection layer including a binding amount of a known binding agent." This reference does not anticipate Applicants' claims, because it does not teach all of the elements of independent claims 22, from which all other claims depend, claim 44, or new claim 46. In fact, Christian requires essential elements that are in direct conflict with Applicants' invention.

The current claims are drawn to a kit that includes "a stack of membranes, said stack comprising a plurality of membranes **each of which are separable from said stack**, wherein each of said plurality of membranes **has substantially a same affinity for said biomolecules** transferred from said sample . . ." This language was already in pending claim 44, and has now been incorporated into independent claim 22; these same features are in new claim 46. Both of the features that are in bold above are expressly excluded from the teachings of Christian, and

thus this reference cannot anticipate claims 22, 44, or 46. Nor can it anticipate any of the dependent claims, which all incorporate the limitations of claim 22.

One of the required elements of the immunoassay rod described in Christian is that the detection layers are "laminated or otherwise bound" to each other (*see*, page 5, line 4). Thus, the capture "layers" taught by Christian **are not separable** from each other and from the rod/stack. Further means for "adhering the spacer layers [] to the detection layers [] to provide a laminated rod structure" are provided at page 9, lines 4-13. These means include adhesives and thermoplastic materials. Use of such adhesive systems for laminating the layers is also discussed at page 10, lines 7-21, for instance. There is no teaching whatsoever in Christian that the layers can or should be separated from each other. In fact, such separation would obviate the purpose of the technology described in Christian. This reference does not and cannot anticipate the current claims.

Another required element of the immunoassay rod described in Christian is that each layer in the rod includes "a binding amount of a known binding agent. The binding agent is capable of specifically binding with the specific substance being tested for." (*See*, Abstract, lines 6-8, and throughout the reference.) Thus, Christian describes an indirect capture system where each layer of the rod has a binding affinity adapted to capture a specific substance. Thus, the layers of the Christian technology do not have "**substantially a same affinity for said biomolecules** transferred from said sample" as required by Applicants' claims. Use of layers having substantially the same affinity for biomolecules would obviate the purpose of the technology described in Christian, particularly since there is no teaching to separate those layers. This is further demonstration that this reference does not and cannot anticipate the current claims.

Applicants also note that Christian is limited to a system for testing liquid samples, which do not have a defined relatively two dimensional relationships between the biomolecules in the sample. Applicants' current claims require that "the stack of membrane is provided in a configuration adapted to be brought into contact with the tissue section so as to create multiple substantial replicas of the biomolecular content of the tissue section, which substantial replicas

maintain the relative relationship of the biomolecules . . ." This limitation is also not met by the teachings of Christian.

In light of the amendments filed herewith, and the arguments presented above, Applicants request that the rejections based on Christian be withdrawn.

Rejection Based on Nieuwkerk *et al.* (US Pat. 5,438,128)

All of the pending claims have been rejected as allegedly anticipated by Nieuwkerk *et al.* As stated in the Office action, Nieuwkerk *et al.* discloses a device that contains a layered membrane assembly for purification of nucleic acids, where the membranes are functionalized with ion-exchange groups to enable the release of purified nucleic acids. This reference does not anticipate Applicants' claims, because it does not teach all of the elements of claims 22, from which all other claims depend, claim 44, and new claim 46. In fact, Nieuwkerk requires essential elements that are in direct conflict with Applicants' invention.

The current claims are drawn to a kit that includes "a stack of membranes, said stack comprising a plurality of membranes each of which are **separable from said stack** . . . and wherein the stack of membranes is **provided in a configuration adapted to be brought into contact with the tissue section so as to create multiple substantial replicas of the biomolecular content of the tissue section**, which substantial replicas maintain the relative relationship of the biomolecules . . ." The first portion of this language was already in pending claim 44, and has now been incorporated into independent claim 22. The phrase "wherein the stack of membranes is provided in a configuration adapted to be brought into contact with the tissue section so as to create multiple substantial replicas of the biomolecular content of the tissue section, which substantial replicas maintain the relative relationship of the biomolecules" has been added to claims 22 and 44 by this amendment; this phrase is supported throughout the specification, including particularly at page 7, lines 17-26, particularly 24-26; and in Figure 1 (illustrating contact between a tissue section and a stack of membranes). Both of these features are also incorporated in new claim 46.

Both of the features that are in bold in the prior paragraph are excluded, expressly or implicitly, from the teachings of Nieuwkerk *et al.*, and thus this reference cannot anticipate claim 22. Nor can it anticipate any of the dependent claims, which all incorporate the limitations of claim 22.

The stack of membranes described in the Nieuwkerk *et al.* are not intended to be separable from each other, nor is there any teaching or enablement to separate the membranes from each other or the housing in which they are supplied. The membranes in fact are "**attached**" to the housing in which they are supplied (Col. 4, line 68). Thus, the membranes taught by Nieuwkerk *et al.* **are not separable** from each other, as required by the current claims. There is no teaching whatsoever in this reference that the membranes can or should be separated from each other. In fact, such separation would obviate the purpose of the technology described in Nieuwkerk *et al.*, since that technology instead depends on removing captured molecules from the membranes (*see, e.g.*, Col. 3, lines 56-61; Col. 4, lines 11-17 and lines 54-55; and so forth). This reference does not and cannot anticipate the current claims.

Similarly, the devices of Nieuwkerk *et al.* are not provided in a configuration adapted to be brought into contact with a tissue section so as to create multiple substantial replicas of the biomolecular content of the tissue section, which substantial replicas maintain the relative relationship of the biomolecule. The devices of Nieuwkerk *et al.* are adapted only to receive fluid samples, where there is no relative two-dimensional relationship between biomolecules in the sample.

The Office action further indicates that Nieuwkerk *et al.* provides for analysis of "specimens such as human bodily fluids and tissues" (citing Col. 2, lines 64-68). This only partially characterizes the teachings of this reference. In fact, the text continues by noting that the samples must be further prepared by "lysing the cells and/or viruses to obtain the nucleic acids." (Col. 3, lines 1-4.) The purpose of the Nieuwkerk *et al.* devices is to purify nucleic acid molecules; such molecules can only be captured by the membranes of the devices if the cells have been lysed. For instance, the *Summary of the Invention* (at Col. 2, line 29) refers to use of the devices with "crude cell lysates" rather than cells or tissue samples.

The lysis techniques provided as exemplary (for instance, alkaline lysis described at Col. 3, lines 4-8; see also Examples 1 and 2, beginning at Col. 8) are fluid-based lysis techniques that do not result in a sample that contain biomolecules having relative two-dimensional relationships to each other. The device could not be used with such samples. Thus, this reference cannot and does not anticipate the current claims.

In light of the amendments filed herewith, and the arguments presented above, Applicants request that the rejections based on Nieuwkerk be withdrawn.

Rejection Based on Imai *et al.* (US Pat 5,057,438)

All of the pending claims have been rejected as allegedly anticipated by Imai *et al.* As stated at page 5 of the current Office action, Imai *et al.* discloses "a plurality of membranes [] formed with an antigen or antibody" on each of the membranes, wherein the "reaction membranes are superimposed. Antibodies or antigens corresponding to the species located on the membranes" can be allowed to bind, and "[t]he membranes are then separated" so that "detection of the analyte of interest on the membrane occurs on each membrane." (*See*, page 5 of the Office action, citing Col. 8-9 of Imai *et al.*) This reference does not anticipate Applicants' claims, because it does not teach all of the elements of claim 22, from which all other claims depend, claim 44, or new claim 46. In fact, Imai *et al.* requires essential elements that are in direct conflict with Applicants' invention.

A required element of the system described in Imai *et al.* is "a plurality of reaction membranes each having a different species of antibody or antigen . . ." (see, Col. 2, lines 20-21). Thus, similar to the Christian reference above, Imai *et al.* describes an indirect capture system where each membrane layer has a binding affinity adapted to capture a specific antibody of antigen. Thus, the membranes of the Imai *et al.* technology do not have "**substantially a same affinity for said biomolecules** transferred from said sample" as required by Applicants' claims. Use of membranes having substantially the same affinity for biomolecules (as Applicants' use) would obviate the purpose of the technology described in Imai *et al.* This clearly demonstrates that Imai *et al.* does not and cannot anticipate the current claims.

In light of the amendments filed herewith, and the arguments presented above, Applicants request that the rejections based on Nieuwkerk be withdrawn.

Claim Rejections – 35 USC §103(a)

Rejection Based on Christian or Nieuwkerk in view of Hunkapiller *et al.* (US Pat. 6,232,067)

Claims 32, 37, and 38 are alleged to be obvious over Christian or Nieuwkerk in view of Hunkapiller *et al.* The teachings of Christian and Nieuwkerk are discussed above. As stated in the Office action, the only potentially relevant element disclosed by Hunkapiller *et al.* and relied upon by the Office is the use particular types of solid supports for the analysis of polynucleotides. Particularly, Hunkapiller *et al.* provides the following description of "solid supports" at Col. 18, lines 12-19:

> The solid support may be of any of a variety of forms such as beads, sheets, membranes, chips, fiber, and the like. Similarly, the solid support may be formed of any of a number of materials compatible with immobilization of nucleic acids, including, but not limited to, glass or polymers, e.g., polystyrene, polyacrylamide, polycarbonate, polyethylene, polypropylene, agarose, and the like.

No other aspect of this reference appears to relate to Applicants' invention; it does not teach anything regarding the use of multiple stacked membranes that are separable from each other, which is required by claims 32, 37, and 38. Hunkapiller *et al.* does not make up for the deficiencies of Christian and/or Nieuwkerk discussed above. As such, this combination of references does not and cannot render these claims obvious.

In addition, Hunkapiller *et al.* does not (expressly or implicitly, and certainly not inherently) teach the use of a membrane that is less than 30 microns thick (as required by claim 32). This limitation is not taught by either Christian or Nieuwkerk either. Thus, these cited references even in combination do not provide all the elements of the invention of claim 32. The Office action alleges that "where the general conditions of a claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art," citing *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). Applicants traverse the statement

that the general conditions of the claim are disclosed in the prior art, for the reasons stated above. In addition, one of ordinary skill in the art would not have considered it obvious, nor would they have had a reasonable expectation of success, to use a membrane thickness of 30 microns to 4 microns when none of the references teach anything related to such thin membranes, and common commercially available membranes (such as those discussed above in reference to the anticipation rejections) are not supplied in thicknesses anything near this range. Even Christian teaches that "detection layers will be from 0.002 to 0.006 inch thick" (page 9, lines 21-22), which equates to about 50 to 150 microns thick (1 micron = 0.00003937 inch). This is substantially thicker than the currently claimed 4-30 microns.

In light of the arguments presented above, Applicants request that this obviousness rejection of claims 32, 37, and 38 be withdrawn.

Rejection Based on Christian or Nieuwkerk *et al.* in view of Pipas *et al.* (US Pat. 6,168,929 B1)

Claim 26 is alleged to be obvious over Christian or Nieuwkerk in view of Pipas *et al.* The teachings of Christian and Nieuwkerk are discussed above. As stated at page 7 of the Office action, Pipas *et al.* discloses "use of an antibody cocktail to probe analytes blotted onto PVDF membranes." As stated in the Office action, the only potentially relevant element disclosed by Pipas *et al.* and relied upon by the Office is the use of such antibody cocktails. No other aspect of this reference appears to relate to Applicants' invention; it does not teach anything regarding the use of multiple stacked membranes that are separable from each other, which is required by claim 26. Pipas *et al.* does not make up for the deficiencies of Christian and/or Nieuwkerk discussed above. As such, this combination of references does not and cannot render this claim obvious.

In light of the arguments presented above, Applicants request that this obviousness rejection of claim 26 be withdrawn.

Provisional Obviousness-type Double Patenting

Claims 22-28 and 32-44 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 92-100 in US

2004/0081979 A1 (the published document corresponding to US 10/432,423; Attorney Docket No. 6457-65767). Applicants note that a Response to Restriction Requirement in US 10/432,423 was submitted to the Office on July 28, 2004; that Response cancelled claims 92-100. Applicants therefore request that the provisional obviousness-type double patenting rejection be withdrawn as moot.

Conclusions

Based on the foregoing amendments and arguments, the claims are in condition for allowance and notification to this effect is requested. If for any reason the Examiner believes that a telephone conference would expedite allowance of these claims, please telephone the undersigned at (503) 226-7391.

Respectfully submitted,

KLARQUIST SPARKMAN, LLP

By _____
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121 S.W. Salmon Street
Portland, Oregon 97204
Telephone: (503) 226-7391
Facsimile: (503) 228-9446

BioTrace™ NT Nitrocellulose Transfer Membrane

Media
Nitrocellulose

Pore Size
0.2 µm

Typical Thickness
145 µm (5.7 mils)

Protein Binding Capacity
209 µg/cm^2

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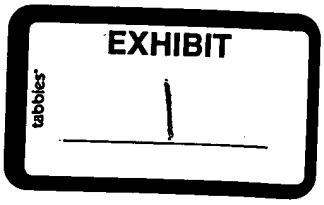
*US List Price applies only to products delivered within the US. These prices do not reflect duties, taxes or tariffs, which may apply to purchases made outside of the US. See our General Limited Warranty.



FP Vericel™ (PVDF) Membrane Disc Filters

Description | Applications | Specifications | Ordering Information

redlineFilter Media
Hydrophilic polyvinylidene fluoride (PVDF)

Pore Size
0.2 and 0.45 µm

Typical Thickness
0.2 µm: 140 µm (5.5 mils)
0.45 µm: 127 µm (5.0 mils)

Typical Water Flow Rate
mL/min/cm^2 at 0.9 bar (13.5 psi)
0.2 µm: 5
0.45 µm: 26

Maximum Operating Temperature - Water
100 °C (212 °F)

Minimum Bubble Point - Water
0.2 µm: 2.3 bar (34 psi)
0.45 µm: 1.1 bar (16 psi)

Sterilization
Provided non-sterile; autoclavable if desired

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*US List Price applies only to products delivered within the US. These prices do not reflect duties, taxes or tariffs, which may apply to purchases made outside of the US. See our General Limited Warranty.

EXHIBIT tabbies 2

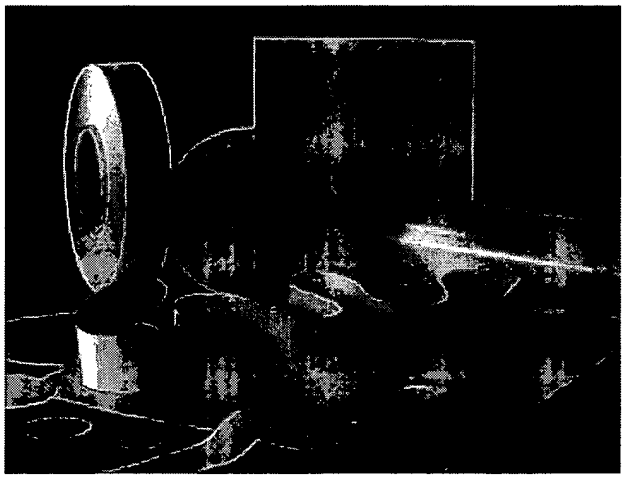
PALL Life Sciences

Membranes for Transfer and Immobilization

BioTrace™, Biodyne®, FluoroTrans® and UltraBind™ Membranes

A variety of membrane chemistries for sensitive detection and consistent results in all applications and detection systems

- **BioTrace NT membrane**—pure unsupported nitrocellulose is used for colony and plaque lifts and has low burn-through in protein transfer applications.
- **BioTrace PVDF membrane**—versatile membrane with broad chemical resistance, ideally suited for protein transfers.
- **Biodyne membranes**—intrinsically hydrophilic Nylon 6,6 membranes provide high sensitivity and low background for enhanced resolution. Ideal for nucleic acid blots and protein ELISA tests.
- **FluoroTrans PVDF membranes**—increased sensitivity, ideal for a wide variety of protein analysis applications including sequencing and western transfers.



- **UltraBind membrane**—affinity membrane is recommended for covalent protein binding.

Applications

- Nucleic acid and protein transfer and detection:
  - Northern, Southern, and Western transfers
  - Colony and plaque lifts
  - Replica plating
  - Dot/slot blots
  - DNA fingerprinting
- Protein sequencing
- Solid phase ELISAs
- Affinity separations
- Macroarrays
- Microarrays

Ordering Information

		Product Numbers										
Description	Packaging	BioTrace NT Membrane	BioTrace PVDF Membrane	Biodyne A Membrane, 0.2 µm	Biodyne A Membrane, 0.45 µm	Biodyne A Membrane, 1.2 µm	Biodyne B Membrane, 0.45 µm	Biodyne C Membrane, 0.45 µm	Biodyne Plus Membrane, 0.45 µm	FluoroTrans W Membrane 0.2 µm	FluoroTrans Membrane 0.2 µm	UltraBind Membrane 0.45 µm
82 mm discs	50/pkg	66487			60102		60202	60316	60402			
85 mm discs	50/pkg	66595			60103		60203	60317	60403			
132 mm discs	50/pkg	66518			60104		60204	60318	60404			
137 mm discs	50/pkg	66488			60105		60205	60319	60405			
7 x 8.5 cm sheets	10/pkg	66593	66594		60101		60201	60315	60401		PVM020C-160	
7 x 9 cm sheets	10/pkg									BSP0158		
8.5 x 9 cm sheets	20/pkg										PVM020C-195	
10 x 15 cm sheets	10/pkg									BSP0157	PVM020C1015	
13 x 14 cm sheets	10/pkg										PVM020C-196	
20 x 20 cm sheets	10/pkg	66489	66542		60100		60200	60314	60400	BSP0159	PVM020C2020	66544
30 cm x 3 m roll	1/pkg	66485	66543	60113	60106	60108	60207	60320	60406			66545
20 cm x 1 m roll	1/pkg		66547				60209					
20 cm x 3 m roll	1/pkg				60120		60208					
3.3 m roll	1/pkg									BSP0161	PVM020C-099	

In addition to standard sizes, these membranes can be cut to size to suit your specifications. For information on special-sized cuts, call your local Pall Life Sciences office.

Filtration. Separation. Solution.SM

EXHIBIT 3 tabbies

Transfer and Affinity Membrane Selection Guide

Pall Life Sciences offers membranes for use in transfer and immobilization procedures. These membranes can be used for nucleic acid and protein applications and are compatible with radioactive, as well as nonradioactive detection systems.

Product	Biodyne® A Membrane	Biodyne B/Plus Membrane	Biodyne C Membrane
Description	Amphoteric Nylon 6,6	Positively-charged Nylon 6,6	Negatively-charged Nylon 6,6
Works best for:	Colony/Plaque Lifts, DNA and RNA Transfers	DNA and RNA Transfers, Multiple Reprobings	Reverse Dot Blots
Also suited for:	Gene Probe Assays, DNA Fingerprinting, Nucleic Acid Dot/Slot Blots, Replica Plating, ELISAs	DNA Fingerprinting, Nucleic Acid Dot/Slot Blots, Colony/Plaque Lifts (Biodyne B membrane), Replica Plating (Biodyne B membrane)	Protein Immobilization, Affinity Purification, ELISAs
Advantages	– High sensitivity – Low background – Net charge can be controlled by changing pH – Ability to strip and reprobe	– Positive charge over broad pH range – Highest sensitivity for nucleic acid applications (Biodyne B membrane) – Ability to strip and reprobe	– Negative charge over broad pH range – Surface carboxyl groups can be derivatized – Ability to strip and reprobe
Binding Interaction	Hydrophobic & Electrostatic	Hydrophobic & Electrostatic	Hydrophobic & Electrostatic
Method of Immobilization	UV Crosslink Baking	Can be baked or UV crosslinked, although not required	Derivatization
Detection Methods	Radiolabeled Probes, Enzyme-antibody Conjugates – Chemiluminescent – Chromogenic	Radiolabeled Probes, Enzyme-antibody Conjugates – Chemiluminescent – Chromogenic – Chemifluorescent (Biodyne Plus Membrane)	Radiolabeled Probes, Enzyme-antibody Conjugates – Chromogenic

Product	BioTrace™ NT Membrane	BioTrace PVDF Membrane	FluoroTrans® Membrane	UltraBind™ Membrane
Description	100% Pure Nitrocellulose	Polyvinylidene Fluoride	Polyvinylidene Fluoride	Modified Polyethersulfone
Works best for:	Colony/Plaque Lifts	Protein Transfers	Western Transfers (FluoroTrans W) N-terminal Protein Sequencing (FluoroTrans PVDF)	Solid-phase ELISAs
Also suited for:	Nucleic Acid and Protein Transfers, Protein Dot/Slot Blots	Protein Dot/Slot Blots		Affinity Chromatography, Hybridoma Screening
Advantages	– Excellent strength – No support fabric – No detergents added – 100% pure nitrocellulose	– Chemical resistance – No discoloration – Nonflammable – High strength	– Strong protein binding – Sensitive detection – Very low burn-through – Good chemical compatibility	– Covalent binding – No preactivation required – High protein-binding capacity
Binding Interaction	Hydrophobic & Electrostatic	Hydrophobic	Hydrophobic	Covalent
Method of Immobilization	UV Crosslink Baking (Vacuum Oven)			Direct Spotting Perfusion
Detection Methods	Radiolabeled Probes, Direct Stain, Fluorescence, Enzyme-antibody Conjugates – Chemiluminescent – Chromogenic	Direct Stain, Enzyme-antibody Conjugates – Chemiluminescent – Chromogenic	Direct Stain with Coomaisse blue, Amido black, Ponceau S, and colloidal gold (FluoroTrans W membrane). Enzyme-antibody Conjugates – Chemiluminescent – Chromogenic	Radiolabeled Probes, Enzyme-antibody Conjugates – Chromogenic

Biodyne® Transfer Membranes

- High sensitivity and low background for enhanced detection and resolution.
- Do not crack, shrink, or tear when subjected to multiple cycles of hybridization, stripping, and reprobing.
- Intrinsically hydrophilic for easy wetting.
- Superior performance with radioactive (Biodyne B membrane) and nonradioactive (Biodyne A membrane) detection systems.
- Ideal for nucleic acid detection.

Applications

Four chemistries provide versatile adsorption properties:

1. Biodyne A Membrane: Amphoteric Nylon 6,6.
   Membrane zeta potential can be modulated by changes in pH. Ideal for single probe or multiple rehybridizations, and applications where background is troublesome.
2. Biodyne B Membrane: Positively-charged Nylon 6,6.
   Pore surfaces are populated by a high density of quaternary ammonium groups. Our highest sensitivity nylon membrane for nucleic acid applications.
3. Biodyne C Membrane: Negatively-charged Nylon 6,6.
   Can be derivatized by coupling reactions through the carboxyl groups on the pore surfaces.
4. Biodyne Plus Membrane: Positively-charged Nylon 6,6 with an extremely high isoelectric point.
   With certain nonradioactive detection systems, it is more sensitive than Biodyne A membrane while exhibiting lower background than Biodyne B membrane.

Specifications

Media
Nylon 6,6

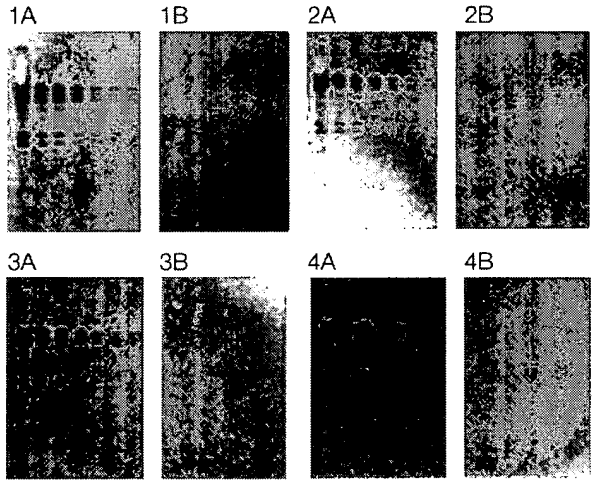
Typical Thickness
6.0 mils ± 0.5 mils

Pore Sizes
0.2, 0.45, or 1.2 µm

Solvent Compatibility
Resistant to common solvents such as acetone, alcohol, chlorinated aliphatic hydrocarbons, formamide, 2 M NaOH, DMSO, and dimethylformamide.
Not compatible with concentrated formic acid (> 50%), HCl (> 4 M), oxidizing agents, and long exposures (days to weeks) at pH < 2.

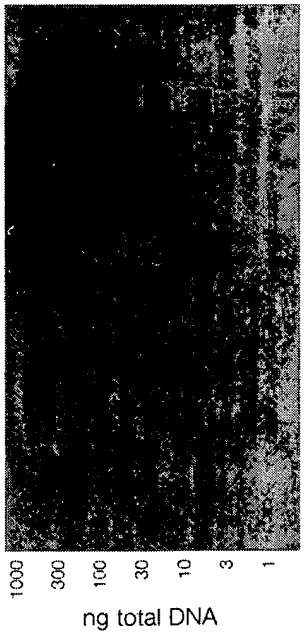
Performance

Biodyne B Membrane Withstands Multiple Cycles of Stripping and Reprobing



Lambda-Hind II DNA fragments were separated in an agarose gel and transferred to Biodyne B membrane using the Pall Improved Alkaline Transfer method. The blot was stripped completely and reprobed four times without loss of signal intensity. Bands were detected using a chemiluminescent detection system.
Panels A (1A - 4A): blot after (re)probing
Panels B (1B - 4B): blot after stripping, prior to (re)probing

Superior Fluorescent Detection of DNA Using Biodyne Plus Membrane



Dilutions of Hind III-digested I-DNA (1,000-1 ng/lane) were separated in an agarose gel and transferred to Biodyne Plus membrane. Signal was generated using a fluorescein-labeled probe, antifluorescein-alkaline phosphatase conjugate, and precipitating substrate. The image was generated by scanning the blot with a FluorImager® system.

BioTrace™ PVDF Transfer Membrane

- Versatile membrane for nucleic acid and protein transfers.
- Broad compatibility with commonly-used solvents.
- Low background with chemiluminescent detection systems.

Applications

- Western transfers
- Southern transfers

Specifications

Media
Polyvinylidene fluoride

Typical Thickness
147 µm (5.8 mils)

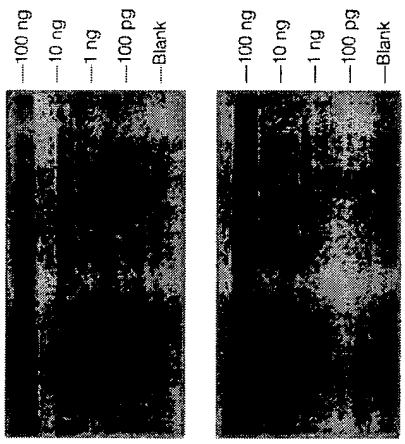
Pore Size
0.45 µm

Tensile Strength
28 bar (410 psi)

Solvent Compatibility
Resistant to methanol, phenol, and chloroform. Also resistant to 10% dimethyl sulfoxide, 15% acetic acid, 70% formic acid, 25% triethylamine, 1 N NaOH, and 1 N KOH.

Performance

Western Transfer to BioTrace PVDF Membrane



Serial dilutions of E. coli lysates were transferred from a 10 - 20% gradient gel to BioTrace PVDF and a competitive PVDF membrane, then probed with rabbit anti-E. coli antibodies. Proteins were visualized using peroxidase-conjugated goat anti-rabbit antibodies and 4-chloro-1-naphthol substrate solution.

PALL Life Sciences

BioTrace™ NT Transfer Membrane

- Pure unsupported nitrocellulose membrane is ideal for colony/plaque lifts and protein transfers.
- Strong and durable, less likely to tear or crack than competitor nitrocellulose.
- High binding capacity for proteins and nucleic acids.
- Lower protein burn-through than competitors in electrophoretic transfers.

Applications

- Colony/plaque lifts
- Protein transfers

Specifications

Media
Nitrocellulose

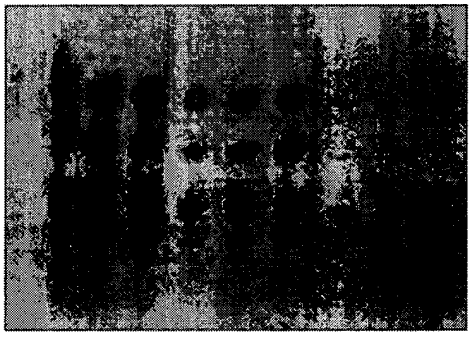
Pore Size
0.2 µm

Typical Thickness
145 µm (5.7 mils)

Protein Binding Capacity
209 µg/cm²

Performance

BioTrace NT Membranes Exhibit Low Protein Burn-through



Prestained proteins were separated in a polyacrylamide gel and electrophoretically transferred to the indicated nitrocellulose membranes. A double layer of membrane was used, one directly against the gel, followed by the second layer. Signal intensity on the second layer is indicative of burn-through, which can lead to loss of signal.

FluoroTrans® PVDF Membrane

- Sensitive protein detection with low background and very low burn-through.
- Membranes provide high surface area for strong hydrophobic interactons and typically adsorb 50% more protein than nylon or nitrocellulose.
- FluoroTrans W membrane is optimized for Western transfer applications.
- FluoroTrans PVDF membrane is optimized for N-terminal protein sequencing.

Applications

FluoroTrans W Membrane:

- Western transfers
- Southern transfers

FluoroTrans Membrane:

- N-terminal protein sequencing

- FluoroTrans media have high tensile strength and will not tear, crack, or curl during handling. This allows for easy removal of target bands for protein sequencing applications.

Specifications

Media
Hydrophobic polyvinylidene fluoride

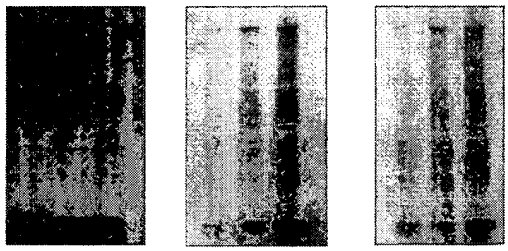
Pore Size
0.2 µm

Chemical Compatibility
Resistant to acetone, DMSO, dimethyl formamide, methanol, trifluoroacetic acid, and triethylamine.

Performance

FluoroTrans Membrane has Excellent Sensitivity, Signal, and Background in Western Transfers

Western Transfers to PVDF Membranes



FluoroTrans PVDF Membrane | FluoroTrans W Membrane | Competitor PVDF Membrane

Rabbit reticulocyte lysate (Amersham) was loaded in lanes of polyacrylamide gels at f.s., 1/3 and 1/10 dilutions. After electrophoresis, proteins were transferred to membranes. Membranes were stained with 0.1% Amido Black, 45% methanol, 2% acetic acid for 4 minutes and were then destained for 5 minutes with two changes of 90% methanol, 2% acetic acid. Stained membranes were rinsed in water and air dried.

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UltraBind™ Affinity Membrane

- Modified polyethersulfone (PES) membrane for covalent protein binding.
- Proteins can be efficiently attached without prior membrane derivitization.

Applications

- ELISA
- Affinity separation

Specifications

Media
Modified polyethersulfone with aldehyde surface chemistry

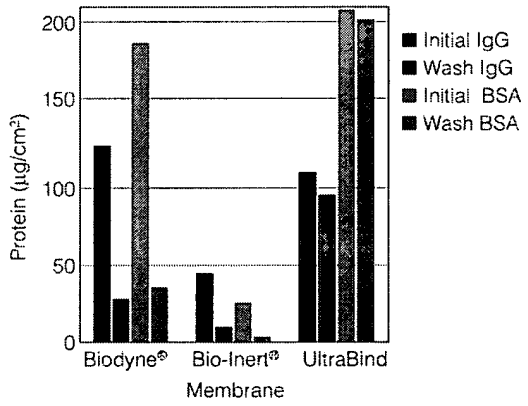
Pore Size
0.45 µm

Typical Thickness
152 µm (6 mils)

Typical IgG Binding Capacity
135 µg/cm²

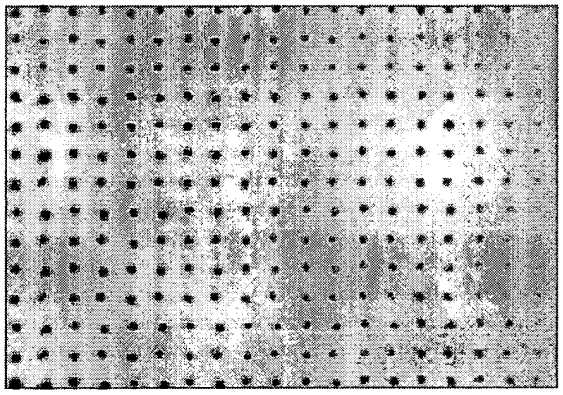
Performance

UltraBind Membrane Binds and Retains Proteins



Membrane discs (13 mm) were soaked in a protein solution and washed to determine the capacity and strength of protein binding. Discs were soaked in radioactively labeled IgG and BSA (200 g unlabeled protein with 100,000 cpm of ^{125}I-labeled tracer) for 60 minutes with agitation, rinsed, and either read in a gamma counter or stripped using a 1% SDS/2 M Urea wash. Biodyne B membrane (charged nylon transfer membrane) and Bio-Inert membrane (modified Nylon 6,6 membrane) were used as high and low binding capacity controls respectively. UltraBind membrane efficiently bound protein and retained it after the SDS/Urea wash.

Antigen Detection (dot blot ELISA) with UltraBind Membrane



Dilutions of human serum albumin (hSA) ranging 2000 to 0.5 pg/spot were applied to UltraBind membrane using a 96-pin transfer tool on a Matrix PlateMate® Liquid Handling Station. The membrane was then blocked with 0.5% Hammerstein-grade casein in PBS. hSA was detected with rabbit anti-hSA antibody followed with alkaline phosphatase conjugated goat anti-rabbit IgG. Signal was generated by reaction with BCIP/NBT substrate allowing detection of as little as 0.5 pg hSA.

Complementary Products

- **Centrifugal Devices** provide precise, rapid processing of the following sample volumes:

Device	Sample Volumes
Nanosep® Device	50 to 500 µL
Microsep™ Device	500 µL to 3.5 mL
Macrosep® Device	1 mL to 15 mL
Jumbosep™ Device	15 mL to 60 mL

- **AcroWell™ 96- and 384-well Filter Plates** with BioTrace Membranes exhibit high binding capacities for proteins and nucleic acids.
- **AcroPrep™ 96- and 384-well Filter Plates** can be used for a variety of molecular biology, combinatorial chemistry, and screening applications.
- **Vivid™ Gene Array Slides** feature a unique membrane construction that allows high signal-to-noise ratios, requires less template, and provides consistent results. Protocols are easy to follow with simple immobilization steps.

Technical Literature

- Discover Endless Potential: Products for Genomics, Proteomics, and Drug Discovery Brochure, Pall Life Sciences, PN33286
- Transfer and Detection Procedures for Pall Life Sciences Membranes and Kits, Pall Life Sciences, PN33167
- Explore the Possibilities: High Throughput Separation, Purification, and Detection Technologies Brochure, Pall Life Sciences, PN33252

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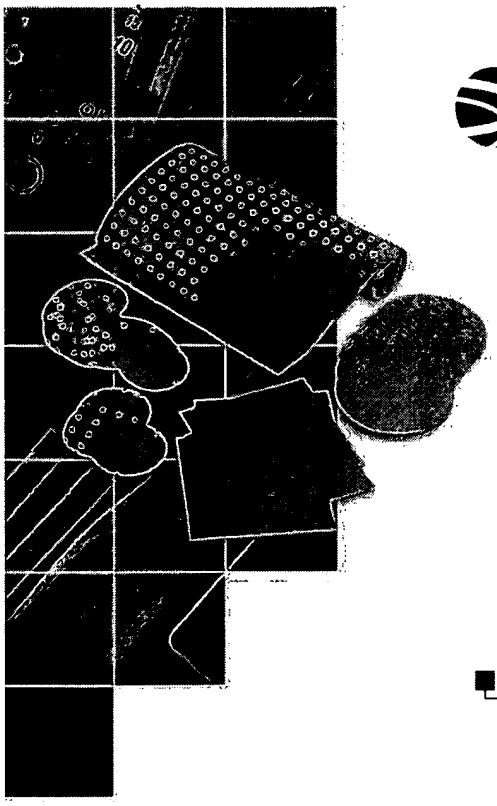

OSMONICS
Filtration and Separations Group | ENGINEERING PURITY

Magna™ PVDF (Polyvinylidene Flouride)

Magna™ PVDF (Polyvinylidene Fluoride) membrane is a naturally hydrophobic, unsupported transfer membrane. It has a high binding capacity and low backgrounds and is ideal for use in protein binding applications such as western blots, solid phase assays and immuno blotting procedures.

Applications

Western blots

Immunoblotting

Solid phase assays

Amino acid or protein analyses

Features and Benefits

Magna PVDF membrane has many features that make it ideal for use in protein binding applications such as western blots, solid phase assays and immuno blotting procedures.

Superior strength	Can withstand aggressive handling or be used with automated equipment without breaking or tearing.
Low extractables	Ensures tests will be clean with consistent results.
Exceptional sensitivity	Detects low-level components.
Hydrophobic	Resists water.
Lot-to-lot consistency	Quality checks ensure consistent binding for dependable results every time.
High binding capacity	Binds a wide range of fragment sizes.
High range of chemical compatibility	Resistant to most commonly used chemicals and chemically aggressive solvents.

Exceptional Strength and Chemical Compatibility

Produced through a proprietary manufacturing process, Magna PVDF membrane meets rigorous quality standards throughout every step of production. This process generates consistent lot-to-lot binding among the membranes and ensures product uniformity.

Magna PVDF is a naturally hydrophobic, unsupported transfer membrane. It has a high binding capacity, which prevents protein from passing through the membrane, and a low background that provides for an excellent signal-noise ratio. It also has exceptional tensile strength, preventing it from cracking, tearing, breaking or curling.

This membrane also has a high chemical compatibility, which is important when used with common stains such as, Amido Black, Colloidal Gold, Coomassie Blue, India Ink and Ponceau-S. Magna PVDF will not degrade, distort or shrink when a high concentration of methanol is used for destaining.

Its exceptional strength, high binding capacity and chemical compatibility make Magna PVDF ideal for use in western blots, immunoblotting, and solid phase assays.

Flexability for OEM Requirements

Magna PVDF membrane is available in rolls up to 30 cm (12 inches) wide, as well as sheets and cut discs that can be customized to meet your application and size requirements. Because the Magna PVDF membrane is manufactured on-site in Osmonics facilities, all customization is easy and cost-effective.

Product Characteristics

Thickness .....140 – 250 µm
Protein Binding .....125 µg/cm² for Immunoglobulins
Pore Size Range .....0.2 and 0.45 µm

For more information about this product please visit the "Shop for OEM Products" section online at www.osmolabstore.com or email us at oemmembranes@osmonics.com.

For technical support, pricing and availability please contact the Osmonics Lab Store Help Desk

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